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PHAGOCYTOSIS AND OPSONINS IN THE LOWER ANIMALS.*

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THE discovery of opsonin by Wright and Douglas has been a great stimulus to the study of the finer mechanism of phagocytosis. This phenomenon is now receiving much attention, so far as concerns the leucocytes and serum of many warm-blooded animals, but nothing seems to have been done in the case of cold-blooded animals. During the past summer we made a study of the mechanism of phagocytosis by the leucocytes of a number of species of the lower animals, and the results of this study are given in the following tables.

In nearly all instances the blood was taken directly from the heart by means of a pipette and mixed immediately with half its volume of one per cent sodium-citrate solution. In the case of blood with serum of high osmotic pressure, enough sodium chloride was added to the sodium-citrate solution to bring the osmotic pressure of the solution approximately up to that of the serum. Whenever the corpuscles were washed sodium-chloride solution having an osmotic pressure approximately equal to that of the serum was used. Frequently it was found necessary to add a trace of sodium citrate to the sodium-chloride solution to prevent clotting of the corpuscles. The mixtures of corpuscles and bacterial suspension were kept at room temperature (summer) for one hour, then smears were made and stained, and the average number of bacteria ingested by each leucocyte determined by counts in 20 or more leucocytes. Sensitization of bacteria was affected by suspending them in the serum for 30 minutes, centrifugating to remove the serum, and washing the bacteria once or twice in a large quantity of NaCl solution. In the sensitization experiments great difficulties were sometimes encountered by the firm agglutination of the bacteria.

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Table 1 shows that the leucocytes in the blood of nearly all species used ingest the ordinary pathogenic bacteria in large numbers. Not

TABLE 1.
PHAGOCYTOSIS OF BACTERIA BY LEUCOCYTES IN BLOOD OF VARIOUS COLD-BLOODED ANIMALS.

	Staphylo- coccus	Strepto- coccus	Pneumo- coccus	Coli communis
Snapping turtle (<i>Chelydra serpentina</i>).....	20+	20+	20	20+
Painted turtle (<i>Chrysemys picta</i>).....	11	14	1	15
Bullfrog (<i>Rana</i>).....	40	13	..	30
Squeteague (<i>Labrus squeteague</i>).....	..	9.9	..	11
Flounder (<i>Pseudopleuronectes Americanus</i>).....	..	7.5	..	4
Conger eel (<i>Conger oceanicus</i>).....	6.6	11	1.6	16
Dogfish (<i>Mustelus canis</i>).....	25	4	..	6
Sand Shark.....	20	12	..	+
Skate (<i>Raja erinacea</i>).....	0	0
King crab (<i>Limulus</i>).....	..	8.7
Clam (<i>Venus</i>).....	30	31

all leucocytes in the smears showed phagocytosis and in the counts those not engaged in phagocytosis were usually disregarded. This was found necessary because there are some leucocytes in these animals which appear never to act as phagocytes and it is extremely difficult to distinguish these from phagocytic cells. In three experiments with the blood of the summer skate no phagocytosis was obtained.

Table 2 shows that there is no phagocytosis of untreated staphylococci by the washed leucocytes of these animals, but there is phagocy-

TABLE 2.
THE IMPORTANCE OF SERUM IN PHAGOCYTOSIS OF BACTERIA BY THE LEUCOCYTES OF COLD-BLOODED ANIMALS.

	Phagocy- tosis
Washed dogfish corp. + Staphylococcus.....	0
" " " + " + dogfish serum.....	25
" " " + " sensitized in dogfish serum.....	20
Washed sand-shark corp. + ".....	0
" " " + " sensitized in shark serum.....	20
Washed snapping-turtle corp. + ".....	1
" " " + " + turtle serum.....	20
Washed frog corp. + ".....	2.7
" " " + " + frog serum.....	40

tosis in these mixtures if normal serum is added to them. Furthermore, there is good phagocytosis by the washed leucocytes if the cocci are sensitized in the serum before they are added to the suspension of leucocytes; that is, the action of the serum on the cocci is essential before phagocytosis can take place. In the sensitization it is not necessary that the serum used comes from an animal of the same

species as that furnishing the leucocytes or from a closely related species. Table 3 shows that sensitization is very successful also with

TABLE 3.
PHAGOCYTOSIS OF BACTERIA SENSITIZED BY HETEROLOGOUS SERA.

				Phagocy- tosis
Washed human corp.	+	Staphylococcus sensitized in human serum.....		17.
" " "	+	" " Limulus serum.....		7.7
" " "	+	" " sand-shark serum.....		9.0
" " "	+	" " snapping-turtle serum.....		15.0
" " "	+	" " Phascolosoma serum.....		9.9
" " "	+	" " Holothurian serum.....		14.5
" " "	+	" " Arbacia serum.....		20.0
" " "	+	" (not sensitized).....		1.8
Washed dogfish corp.	+	" sensitized in dogfish serum.....		25.0
" " "	+	" " sand-shark serum.....		17.8
" " "	+	" " painted-turtle serum.....		20.0
" " "	+	" " Limulus serum.....		20.0
" " "	+	" " human serum.....		10.0
" " "	+	" (not sensitized).....		0.0
Washed sand-shark corp.	+	" sensitized in sand-shark serum.....		20.0
" " "	+	" " dogfish serum.....		20.0
" " "	+	" " painted-turtle serum.....		20.0
" " "	+	" (not sensitized).....		0.0

heterologous sera. Indeed, the serum of some of the very lowest forms of animal life, such as the sea-urchin, may be used to sensitize staphylococci for phagocytosis by human leucocytes. Variable results are frequently obtained in these sensitization experiments, which may be due to the fact that some of these sera may agglutinate the cocci very firmly, making phagocytosis practically impossible. Some sera, namely, that of the summer skate, squeteague, flounder, sea-robin, lobster, and spider crab, entirely failed to sensitize the cocci for phagocytosis by human leucocytes. Our experiments have shown that many sera of cold-blooded animals are highly toxic for human leucocytes. If the leucocytes are left for one hour in the serum of conger eel, dogfish, flounder, squeteague, skate, or frog, the nuclei are vacuolated and usually considerably disintegrated. The chromatin is often found in fine shreds scattered throughout the cytoplasm of the cell. In these experiments the sera were diluted with twice their volume of normal salt solution or salt solution and distilled water. The distilled water was used when the serum normally had a much higher osmotic pressure than that of human serum. Heating these sera to 55° C. for 30 minutes destroys the leucotoxin.

Table 4 shows that the opsonin in the sera of cold-blooded animals is a thermolabile substance, being destroyed by heating at 55° C. for

TABLE 4.
THE EFFECT OF HEAT ON OPSONINS OF COLD-BLOODED ANIMALS.

					Phagocy- tosis.
Washed dogfish corp.	+	Staphylococcus.....			0
" " "	+	Colon bacillus.....			0
" " "	+	Staphylococcus+dogfish serum.....			25
" " "	+	" " " heated 55° 30'.....			0.2
" " "	+	Colon bacillus+ " " " heated 55° 30'.....			20
" " "	+	" " " " " heated 55° 30'.....			0.5
" " "	+	Staphylococcus+ Limulus serum.....			20
" " "	+	" " " " " heated 55° 30'.....			0
Washed snapping-turtle corp.	+	" " " " " ".....			1
" " "	+	" " " " " ".....			20
" " "	+	" " " " " " heated 55° 30'.....			2.0
Washed land-shark corp.	+	" " " " " ".....			14.0
" " "	+	" " " " " " heated 60° 30'.....			0
Washed human corp.	+	" " " " " ".....			6.0
" " "	+	" " " " " " heated 50° 15'.....			7.0
" " "	+	" " " " " " heated 55° 15'.....			0.8

30 minutes. Not many experiments were made to determine the exact temperature at which these opsonins are destroyed but in the case of the *Limulus* it was not destroyed at 50° C. in 15 minutes.

CONCLUSIONS.

1. The unwashed leucocytes of many forms of cold-blooded animals readily take up *in vitro* the common forms of bacteria (staphylococci, streptococci, pneumococci, colon bacilli).

2. Washed leucocytes of many of the lower forms do not take up bacteria; but if the latter are sensitized in the homologous or in many heterologous sera they readily undergo phagocytosis by the washed leucocytes.

3. Bacteria sensitized in sera of cold-blooded animals may be readily taken up by the washed corpuscles of warm-blooded animals; and vice versa.

4. Phagocytosis is prevented or greatly inhibited by heating the various sera to 55° C. for 30 minutes.

5. In some cases phagocytosis was not obtained by adding sera of certain animals to heterologous corpuscles. In such mixtures there is evidence that substances are present in the serum which are toxic for the leucocytes and thus may prevent phagocytosis.

6. Phagocytosis in representative forms of all the great groups of animals down to and including the echinoderms (Echinodermata, Mollusca, Vermes, Arthropoda, Vertebrata) seems to be largely dependent on the presence of opsonins in the sera.